Gibson Assembly Protocol (for single insert)

Name: *Date:*

Rationale:

Observations:

Results:

1. Design primers and perform PCR to amplify and append complementary ends to fragments – purify as needed.
2. Calculate amount of vector and insert according to the following formulas:

$$vector: \frac{(.125\ pmol)\left(660\frac{Da}{bp}\right)(bp\ length\ of\ vector\ fragment)}{\left(1000\frac{pmol}{nmol}\right)\left(\frac{ng}{uL}\ concentration\ of\ purified\ vector\right)} = uL\ vector$$

$$insert: \frac{(.375\ pmol)(660\frac{Da}{bp})(bp\ length\ of\ insert\ fragment)}{(1000\frac{pmol}{nmol})(\frac{ng}{uL}concentration\ of\ purified\ insert)} = uL\ insert$$

   NOTE: Daltons (Da) are equivalent to amu, or g/mol. You can check that the units cancel.
3. Calculate the sum of these volumes. If it exceeds 10 uL, scale it down proportionally to 10 uL (don't worry if it's a large decrease – the Gibson assembly can work with as little as .02 pmol total DNA!). If it's less than 10 uL, calculate the amount of ddH_2O to add to bring it up to 10 uL.
4. Pipette these amounts into a small PCR tube, and then add 10 uL of 2X Gibson Assembly Master Mix (located in the 'New Enzyme' box) and mix. The final reaction mix includes (fill in amounts below):
   - _____ uL vector (vector details: _____)
   - _____ uL insert (insert details: _____)
   - _____ uL ddH_2O
   - 10 uL Gibson MM
5. Place in thermal cycler and run the 'iGEM Gibson' protocol, which incubates at 50°C for 1 hour followed by a 4°C hold. Be sure to adjust the sample volume to 20 uL when running.
6. Store on ice if proceeding to transformation (use ≤5 uL) or leave in -20°C freezer.